

Frank Martin

ARS, Salinas, California

- Generation of NGS data for Oomycetes
 - Sharing *Pythium/Phytophythium* data with Hai Nguyen
 - Have additional *Phytophthora* data
 - Welcome collaboration/sharing data from additional Oomycetes
- Assembly of mitochondrial genomes

Oomycete Mitochondrial Genomes Assembled

- Pythium – 29 species, 34 genomes
- Phytophthora – 63 species, 134 genomes
- Downy Mildews – 8 genera, 20 species, 350 genomes
- Salisapilia – 3 species, 3 genomes
- Aphanomyces – 8 species, 27 genomes

With other miscellaneous taxa over 570 mt genomes assembled

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- Assembly of mitochondrial genomes
 - Development of mitochondrial haplotype markers
 - Extraction of genes for phylogenetic analysis
 - Development of a systematic approach diagnostic markers

Systematic Design of Mitochondrial Diagnostic Markers

- Use comparative genomics to identify gene order differences
 - Enhances specificity, annealing temperature less important
 - *Phytophthora*, *Pythium*, *Aphanomyces*
- Identified unique putative *orf* as target for diagnostic assay
 - More effective for downy mildews
 - Currently working with the select agent *Peronosclerospora philippinensis* and other graminicolous DMs

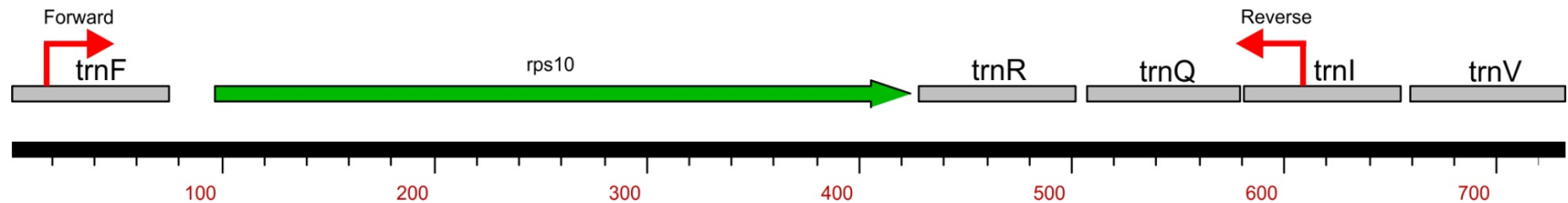
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- Generation of NGS data for Oomycetes
- Assembly of mitochondrial genomes
- Rapid species identification
 - Development of the *rps10* gene as a barcode locus

Barcode for ID and Metagenomics

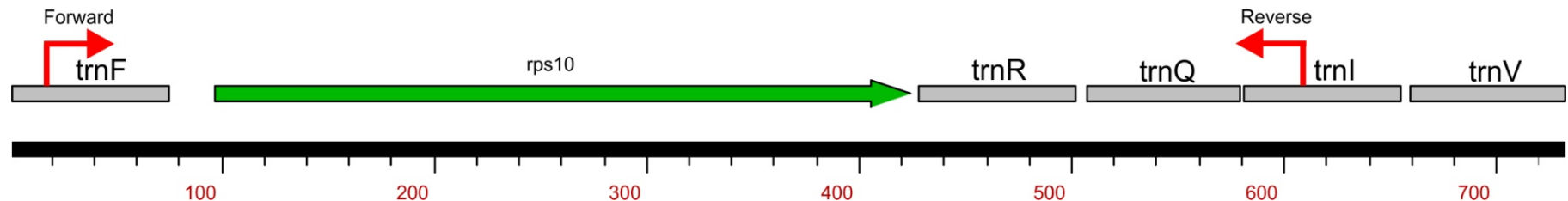
rps10 locus



Amplicon approximately 600 bp
***rps10* gene – 327 bp**

- Highly conserved gene order in oomycetes
 - Not present in plants or Eumycotan fungi
 - Highly conserved primer annealing sites for oomycetes
 - Low/no background from environmental samples

rps10 as Barcode for Isolate Identification



Amplicon approximately 600 bp
***rps10* gene – 327 bp**

- Assembling sequence database to evaluate feasibility
 - Approximately 800 *Phytophthora* sequences representing 150+ taxa
 - A number of sequences from other oomycete taxa included
- Expanding the representation of taxa in collaboration with:
 - Treena Burgess
 - Nik Grunwald
 - JoAnne Crouch
 - Hai Nguyen
 - Hermann Voglmayr
 - Richard Michelmores

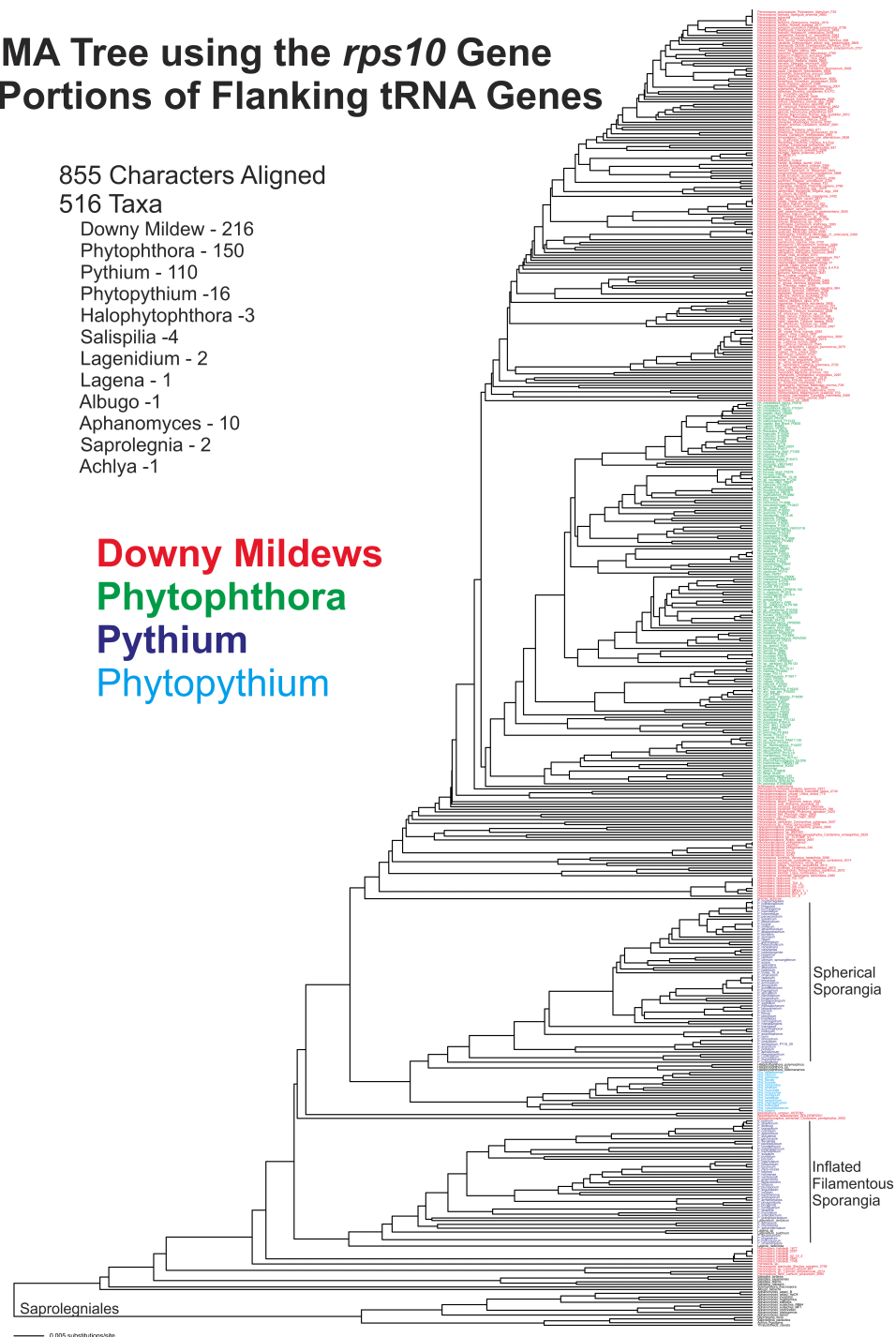
UPGMA Tree using the *rps10* Gene and Portions of Flanking tRNA Genes

855 Characters Aligned

516 Taxa

Downy Mildew - 216
Phytophthora - 150
Pythium - 110
Phytopyhtium -16
Halophytophthora -3
Salispilia -4
Lagenidium - 2
Lagenia - 1
Albugo -1
Aphanomyces - 10
Saprolegnia - 2
Achlya -1

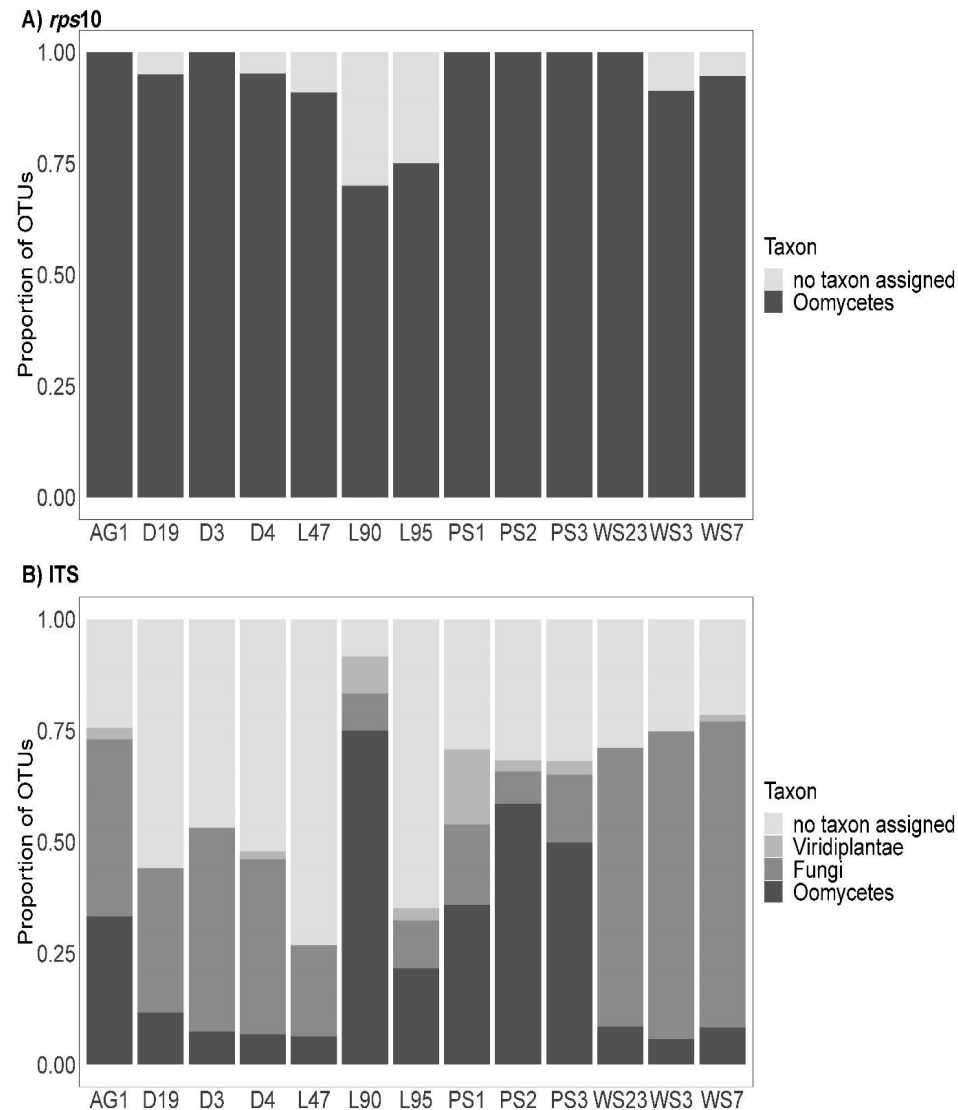
Downy Mildews
Phytophthora
Pythium
Phytopyhtium



rps10 for Metagenomic Studies

Grunwald Lab

Environmental
Samples



rps10 for Metagenomic Studies

Grunwald Lab

- New website named OomyceteDB for metabarcoding resources including primer sequences, lab protocols, and a reference database for taxonomic assignment (<http://oomycetedb.cgrb.oregonstate.edu/>).
- The reference database currently contains the 648 *rps10* sequences for 190 oomycete species.
 - Additional taxa added as *cox1* data to confirm ID is completed
- Currently looking at over 800 isolates to evaluate intraspecific variation

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